

KOUZARIDES
Appl. No. 09/700,417
September 15, 2003

AMENDMENTS TO THE CLAIMS:

This listing of claims will replace all prior versions, and listings, of claims in the application:

1. (Currently Amended) An assay method for an agent which affects E2F acetylation, the method including:

(a) treating an acetylated E2F polypeptide or an acetylated E2F peptide with a test compound, or

(b) treating with a test compound an E2F polypeptide or an acetylated E2F peptide which comprises one or more lysine residues corresponding to those found at positions 117, 120 and 125 in wild-type E2F1, in which polypeptide or peptide one or more of said lysines is not acetylated, or

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(c) bringing into contact a substance which includes a P/CAF polypeptide which acetylates E2F, a substance which includes an E2F polypeptide or an E2F peptide including a site acetylated by P/CAF, and a test compound;

and, following step a, b or c,

(d) determining acetylation of the E2F polypeptide or E2F peptide,

wherein said E2F polypeptide has a sequence selected from the group consisting of the human E2F1, E2F2, E2F3, E2F4 and E2F5 sequences,

said E2F peptide has is a peptide fragment of a sequence selected from the group consisting of the human E2F1, E2F2, E2F3, E2F4 and E2F5 sequences, and;

said P/CAF polypeptide has the sequence of human P/CAF.

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2. (Currently Amended) An assay method for an agent which affects E2F activity, the method including:

- (a) bringing into contact E2F and a test compound; and
- (b) determining E2F activity in the presence and absence of a P/CAF polypeptide which acetylates E2F,

wherein E2F has a sequence selected from the group consisting of the human E2F1, E2F2, E2F3, E2F4 and E2F5 sequences and said P/CAF polypeptide has the sequence of human P/CAF.

3. (Currently Amended) An assay method for an agent which affects E2F activity, the method comprising:

(a) providing an E2F polypeptide which activates transcription from a promoter including an E2F binding site, a test compound, and a reporter construct including a promoter which includes an E2F binding site and which is operably linked to a reporter sequence for transcription thereof, under conditions wherein, in the absence of the test compound being an inhibitor of E2F acetylation, the reporter sequence is transcribed, or

(b) providing an E2F polypeptide which activates transcription from a promoter including an E2F binding site, which polypeptide comprises one or more lysine residues corresponding to those found at positions 117, 120 and 125 in wild-type E2F1,

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and in which polypeptide or peptide one or more of said lysines is not acetylated, a test

compound, and a reporter construct including a promoter which includes an E2F binding site and which is operably linked to a reporter sequence for transcription thereof, under conditions wherein if the test compound promotes acetylation of E2F the reporter sequence is transcribed, or

(c) providing an E2F polypeptide which interacts with P/CAF and activates transcription from a promoter including an E2F binding site, a P/CAF polypeptide which interacts with E2F, a test compound, and a reporter construct including a promoter which includes an E2F binding site and which is operably linked to a reporter sequence for transcription thereof, under conditions wherein, in the absence of the test compound being an inhibitor of interaction between P/CAF and E2F, the reporter sequence is transcribed;

and, following step a, b or c

(d) determining promoter activity,

wherein said E2F polypeptide has a sequence selected from the group consisting of human E2F1, E2F2, E2F3, E2F4 and E2F5 sequence; and said P/CAF polypeptide has the sequence of human P/CAF.

4. (Currently Amended) An assay method for an agent which modulates interaction between P/CAF and E2F, the method including:

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(a) bringing into contact a first substance including a P/CAF polypeptide or a P/CAF peptide, a second substance including an E2F polypeptide or an E2F peptide, and a test compound under conditions in which, if of the test compound does not disrupt the interaction ~~interaction~~ between P/CAF and E2F, the first and second substances interact; and

(b) determining interaction between the first and second substances, wherein said E2F polypeptide has sequence selected from the group consisting of the human E2F1, E2F2, E2F3, E2F4 and E2F5 sequences;

said E2F peptide is a peptide fragment of a sequence selected from the group consisting of the human E2F1, E2F2, E2F3, E2F4 and E2F5 sequences; and

said P/CAF polypeptide has the sequence of human P/CAF.

5. (Currently Amended) An assay method for an agent which affects one or more of (i) ability of E2F to stimulate transcription, (ii) induction of S-phase in cells, (iii) oncogenicity of cells, and/ (iv) induction of apoptosis in cells, the method comprising:

(a) bringing into contact a P/CAF polypeptide and a test compound, and

(b) determining P/CAF acetyltransferase activity;

wherein a test compound which inhibits P/CAF acetyltransferase activity is identified as a candidate said agent,

wherein E2F is selected from the group consisting of human E2F1, E2F2, E2F3, E2F4 and E2F5; and, said P/CAF polypeptide has the sequence of human P/CAF.

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6. (Currently Amended) A method according to claim 5 comprising determining acetylation of E2F by said P/CAF polypeptide.
7. (Original) A method according to claim 5 comprising determining E2F activity.
8. (Previously Presented) A method according to claim 5 wherein a test compound which inhibits P/CAF acetyltransferase activity is further tested for ability to affect one or more of (i) ability of E2F to stimulate transcription, (ii) induction of S-phase in cells, (iii) oncogenicity of cells, and (iv) induction of apoptosis in cells.
9. (Currently Amended) An assay method for an agent which interacts with a region of P/CAF or a region of E2F, which region of P/CAF interacts with E2F and which region of E2F interacts with P/CAF, a said agent which interacts with a said region being a candidate modulator of interaction between P/CAF and E2F, the method including:
- (a) bringing into contact a substance which includes a P/CAF peptide which interacts with E2F, or which includes an E2F peptide which interacts with P/CAF, and a test compound; and
 - (b) determining interaction between said substance and the test compound,
wherein said E2F polypeptide has a sequence selected from the group consisting of the human E2F1, E2F2, E2F3, E2F4 and E2F5 sequences;

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said E2F peptide is a peptide fragment of a sequence selected from the group

consisting of human E2F1, E2F2, E2F3, E2F4 and E2F5 sequences; and,

said P/CAF polypeptide has the sequence of human P/CAF.

10. (Previously Presented) A method according to any one of claims 1, 2, 3, 4, 5 and 9 further comprising formulating a said agent into a composition comprising at least one additional component.

11-26 (Cancelled).